

ImmunoTools *special* Award 2016



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Evaluation of the *in vivo* treatment with DNA demethylating agents on the development and effector function of CD8T cells

Cancer is a group of diseases that begin when an altered cell starts to divide uncontrollably, invading tissues and impairing the function of a given system of the body. Importantly, cancer is the second leading cause of death worldwide. Therefore, much attention has been given to better understand the molecular mechanisms of tumorigenesis and the immune response to cancer cells, both of which aiming at the development of new ways to treat patients.

One of the most promising and novel targets refers to the epigenetic changes observed in cancer cells. The current belief is that DNA demethylating agents act by re-inducing the transcription of tumor suppressor genes otherwise silenced by epigenetic modifications. However, it has been recently demonstrated that DNA demethylation also induces the expression of endogenous dsRNA and the activation of antiviral pathways, a process that was named "viral mimicry". This process contributes to the activation of a long-lasting anti-tumor immune response.

However, the impact of *in vivo* administration of DNA demethylation agents on CD8 T cell expansion, differentiation and function is still poorly understood. Therefore, our project aims to investigate the effect of *in vivo* administration of 5-Azacytidine on the development and function of the effector CD8 T cells. We will use the well-established system of immunization with recombinant adenoviral vectors, which raises a strong CD8 T cell response, along with ELISPOT (to measure the frequency of antigen-specific CD8 T cell response) and the *in vivo* cytotoxic assay (to evaluate the *in vivo* elimination of antigen-specific CD8 T cell targets).

ImmunoTools reagents are definitely of great help for the rapid development of our project. We will use a combination of antibodies and ELISA kits, as well as recombinant mouse cytokines to unravel the type of CD8T cell response raised in mice treated with demethylating agents.

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includes 25 reagents

Multicolour combinations anti-human:

CD3 FITC / CD4 PE

CD3 FITC / CD8 PE

CD4 FITC /CD8 PE /CD45 PerCP

FITC - conjugated anti-mouse Annexin V, CD11b

PE - conjugated anti-mouse CD8a, CD19

PerCP - conjugated anti-mouse

APC - conjugated anti-mouse CD3, CD4, CD25, NK Cells

mouse TNF-alpha ELISA-set for 96 wells, (each 3 reagents)

recombinant mouse cytokines: GM-CSF, IL-1alpha, IL-2, IL-4, IL-6, IL-10, IL-33,
INFg, sCD40L/CD154, sRANKL, TNFa

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